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Original article

C-MET is expressed in the majority of penile squamous cell carcinomas and correlates with polysomy-7 but is not associated with MET oncogene amplification, pertinent histopathologic parameters, or with cancer-specific survival

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ABSTRACT

We assessed c-MET expression and oncogene amplification in a cohort enrolling 92 surgically treated penile squamous cell carcinomas (PSCCs). A tissue microarray was constructed for c-MET immunohistochemistry (IHC) and chromogenic silver in situ hybridization (SISH). Two independent pathologists evaluated IHC by employing the breast cancer scoring rules, and scored the presence of MET oncogene amplification and/or polysomy-7. Eighty study cases (87%) showed c-MET expression. No study case had MET oncogene amplification, but 42 patients (45.7%) had polysomy-7. Polysomy-7 showed a significant positive correlation with c-MET expression ($\rho = 0.323$, $p = 0.002$). Neither c-MET expression nor polysomy-7 was associated with histopathologic parameters or with cancer-specific survival (median post-surgical follow-up 32 months). Our data suggest that the majority of PSCCs exhibit c-MET expression which is not associated with oncogene amplification, but might be attributable to polysomy-7. Further studies should investigate the expression and activation of downstream molecules functionally involved in c-MET pathway signaling, and clarify the so far unresolved role of c-MET inhibitors as potential targeted therapies in PSCCs with metastatic dissemination.

Introduction

Mesenchymal–epithelial transition (MET) is recognized as an oncogene that encodes for an activated version of the hepatocyte growth factor (HGF) tyrosine kinase receptor that plays important roles in cell-cell detachment, cell motility, proliferation, survival, tumor angiogenesis, invasiveness, and metastasis [1–5]. C-MET, the receptor encoded by the MET oncogene, was originally identified in a form rearranged by transfection of DNA from a human osteosarcoma cell line treated *in vitro* with a chemical carcinogen [6,7]. In various human tumors, MET oncogene dependency occurs when the MET tyrosine kinase becomes constitutively active secondary to mutation (found in hereditary papillary renal cell carcinomas and lung cancers) or amplification of the MET oncogene (reported in gastric, esophageal, and lung cancers) at the 7q31 locus [8–11]. Due to these implications, the c-MET signaling pathway has recently attracted considerable interest with respect to the tailored development of c-MET pathway inhibitors (targeted therapies) [12–16]. In particular, two c-MET inhibitors (*PHA-665752* and *PF-2341066*) have shown potency and specificity for inhibiting c-MET activation in a variety of human tumor cells, and several MET tyrosine kinase inhibitors (TKIs) are currently undergoing clinical trials in humans [16–23].

Although the role of c-MET has recently been investigated in a plethora of adenocarcinomas and squamous cell carcinomas (SCCs) from different anatomic sites, c-MET expression profiles and MET oncogene amplification patterns in penile SCCs (PSCCs) have been neglected so far to our knowledge [22,24–26].

This background prompted us to conduct this multi-center study in order to evaluate c-MET expression, MET oncogene amplification status, and polysomy of chromosome 7 (polysomy-7), as well as the associations with pertinent histopathologic parameters

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and cancer-specific survival (CSS) in a study cohort enrolling 92 patients with surgically treated PSCCs histologically characterized by means of a central histopathologic review.

Materials and methods

Study patients

After IRB approval for evaluating CSS by means of death certificates was obtained by the Medical Ethics Committee of the federal state Brandenburg (MEC-No.: 8533/12), a thorough computerized database analysis involving 6 different pathology files affiliated to 6 different Charité-University Medicine Teaching Hospitals in Brandenburg, Germany, was performed, and identified a total of 110 patients surgically treated for PSCCs (27 total and 77 partial penectomies, as well as 6 excisional biopsies). Then, the corresponding archival wax-embedded specimens and conventionally stained histology slides were retrieved from the files.

Processing and handling of the surgical specimens were unique in the 6 involved pathology institutes. Briefly, surgical pathology dissection followed anatomic landmarks with multiple contiguous histologic sections having been taken from all study specimens which were subsequently fixed with neutral formalin for 12 h [27]. In order to reliably establish a diagnosis of verrucous carcinoma (VC), serial sections were cut from the entirely embedded tumor.

Central histopathologic review of the study cases

In order to standardize histologic staging (according to the 7th edition of the TNM classification system) and grading (according to Broders' classification system), all retrieved study slides were histologically re-examined by one clinical pathologist (S.G.) [28,29].

Briefly, basaloid, spindle cell, and any other SCC subtypes harboring any portion of anaplastic cells were considered high-grade, whereas papillary and warty carcinomas, as well as VCs, were regarded as low-grade tumors [30].

Based on conventionally stained sections, the following parameters were recorded during the central histopathologic review: anatomical tumor localization, perineural and lymphovascular invasion (PNI, LVI), tumor thickness (vertical depth of infiltration from the granular cell layer of the adjacent epithelium or from the base of the ulcer to the deepest point of invasion), corpus cavernosum/spongiosum invasion, surgical margin status, koilocytosis, and the histologic pattern of growth as previously reported [31,32]. The presence or absence of tumor-associated eosinophilia ($\geq$10 eosinophilic granulocytes in the peritumoral stroma or $\geq$ 1 eosinophilic granulocyte in the epithelial areas of invasive cancer determined at $\times$400 magnification) was recorded [33].

Tissue microarray (TMA) construction

A TMA (one punch of each tumor with 1.5 mm punch diameter) comprising PSCC tissue from 110 consecutive patients was constructed, and 4 μm thick sections were subsequently cut. Due to tissue loss during sectioning, 92 study patients (median age 68 years, IQR 57–76) remained available for studies. Amongst these 92 study cases, lymph node dissection was performed in 27 individuals based on clinical and/or radiologic evidence of nodal involvement, as well as on individual decisions of the patients concerned. The 65 patients in whom no lymph node dissection was performed (pNx) had clinically unremarkable nodes (cN0) based on palpation and ultrasound examination. None of the 92 study patients received adjuvant chemotherapy and/or radiotherapy.

Immunohistochemistry (IHC) and staining procedure

IHC was performed using an automated staining system (Ventana BenchMark). Following deparaffinization, rehydration, and heat-induced epitope retrieval (CCl mild, Ventana BenchMark), the CONFIRM anti-Total c-MET rabbit monoclonal antibody (Ventana, clone SP44, dilution 1:50) was employed and incubated for 30 min. Adequate positive controls (non-small cell lung and stomach cancer) were run, and negative controls had the primary antibody replaced by buffer.

Chromogenic silver in situ hybridization (SISH)

In order to study MET oncogene amplification status and the frequency of polysomy-7 amongst the 92 study cases, SISH was performed (Ventana Inform Met DNA Probe) as a dual color in situ hybridization using the Inform Met DNA Probe (dark nuclear dots) and Inform chromosome 7 Probe (red nuclear dots) employing the same automated staining system (Ventana BenchMark).

Evaluation of IHC and SISH results

After a few weeks had elapsed since the performance of the central histopathologic review, two independent clinical pathologists (S.G. and A.E.) evaluated c-MET expression detected by means of IHC and, a few weeks later, SISH results by recording the presence of MET oncogene amplification and/or polysomy-7.

Evaluation of IHC was accomplished as recently reported for HER-2 immunostaining evaluation [34]. Briefly, by employing the breast cancer scoring rules and adopting the 10% area cut-off, the study specimens were scored as 0, 1+ (unequivocal intercellular membranous staining confirmed only at $\times$40 objective), 2+ (membranous staining disclosed at medium magnification, $\times$10 objective) or 3+ (unequivocal membranous staining already visible at low magnification, $\times$2.5 objective) (Fig. 1).

Statistical analysis

Frequencies and proportions were assessed for categorical variables, while medians and interquartile ranges (IQRs) were computed for continuously coded variables. The chi-square test was used to test the statistical significance of proportions. Kappa (K)-statistics was employed to assess interobserver reproducibility between both raters. Spearman correlations (ρ) were calculated to evaluate the correlation between c-MET expression and various parameters (age at surgery, histologic growth patterns, pT- and pN-stage, Broders' grade, PNI, LVI, status of surgical margins, koilocytosis, tumor-associated eosinophilia, tumor size and thickness, as well as histologically confirmed invasion of corpus cavernosum, spongiosum, and/or urethra).

Median follow-up was 32 months (IQR: 6–66). CSS was estimated according to the Kaplan–Meier method, and comparisons of the survival curves were made by using the log-rank test. Multivariable analyses for prediction of cancer-specific mortality (CSM) were performed using a Cox proportional hazards regression model, and categoric variable cut points were selected before the data were examined. Adjusted hazard ratios (HRs) were calculated for all covariates using Cox proportional hazards regression analyses with associated p-values. All statistical comparisons were two-sided, and a p-value < 0.05 was considered statistically significant. Data were analyzed using R statistical package (v.2.12.2) and SPSS 19.0 (SPSS Inc. Chicago, IL, USA).